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THE UNIVERSITY OF NORTH CAROLINA
AT
CHAPEL HILL

School of Dentistry
Dental Research Center
Chapel Hill, N.C. 27599-7455

October 26, 1989

Captain Anthony J. Melaragno
Director of Research and Development
Naval Medical Research and Development Command
National Naval Medical Center
Bethesda, MD 20814-5044

Dear Captain Melaragno:

We are pleased that you gave us a little time beyond October 16 to get you our quarterly report on Contract N00014-87-K-0139, (Stain Test Modules for Periodontal Diagnosis.) This has permitted us to find a new use for the positive stain we developed with your support for demonstrating Gram negative bacteria. We had shown earlier that a modification of this silver stain, which Beverly Giammara and Jacob Hanker have patented and is marketed by Sigma Diagnostics, was more useful for demonstrating Pneumocystis carinii (and suggesting the presence of AIDS) than other commercially available techniques.

Over the last few weeks we have shown that this same technique is also very useful for demonstrating Candida albicans in subgingival plaque. We are now comparing the numbers of spores and hyphae present in subgingival plaque samples of AIDS patients with those of the people having periodontal problems who are not HIV-seropositive. It appears that significant counts* of C. albicans cannot be demonstrated in the subgingival plaques of most patients with periodontal disease. So far we have been able to show significant counts of this fungus in 21 of the subgingival plaque smears of the 8 AIDS patients whose samples we have studied. We have seen significant numbers of this fungus only in 6 of the subgingival plaque smears of the 19 HIV-seronegative periodontal patients. The smears of the latter people generally had much lower numbers of spores or hyphae than those of the HIV-seropositive patients (Table 1). It should be realized that the 6 HIV-seronegative patients with significant numbers of candida present in their plaques could be showing immunosuppression by corticosteroids or other therapeutic agents, diabetes or other illnesses.

In addition to staining samples of the subgingival plaques we are culturing aliquots of these samples by two methods. The mycology laboratory of North Carolina Memorial Hospital, under the direction of Dr. Roy L. Hopfer,

*Davenport Index, See Table 1

is culturing one aliquot with a modification of Sabouraud's dextrose agar broth. We are culturing the other aliquot of the plaque samples in our dental clinics with the Microstix^{R}-Candida miniaturized culture test, now marketed for vaginal specimens by the Diagnostics Division of Miles Inc. The latter culture procedure gives a direct color answer by the appearance of brown spots on the culture strip within 24 hours and does not require microscopic examination. We anticipate that this test may eventually be used routinely in our hospital dental clinic and dental school clinics for screening for the presence of C. albicans in subgingival plaque. It is expected that it may also be useful in the dental office to screen the plaques on the under side of prostheses to indicate denture stomatitis. It is felt that the presence of large numbers of C. albicans in the subgingival plaques of homosexuals or IV drug addicts indicated by our microscopic method and the Microstix examination might suggest that they be followed and screened more carefully for the presence or appearance of AIDS. Our microscopic method suggests the presence of C. albicans in 15 minutes whereas the Microstix method takes 24 hours.

Enclosed find approximately 3 dozen light and electron micrographs of C. albicans in subgingival plaque smears of AIDS patients being treated at North Carolina Memorial Hospital by Drs. Phil Webster and Darryl Hamamoto. The samples of subgingival plaques from perio patients in the dental school have been supplied by Drs. E.J. Burkes, Jr. and G.W. Greco. Samples of cultured C. albicans have been supplied by Drs. M.J. Kutcher and R.L. Hopfer. After preliminary discussions, it appears that the use of these tests to look for C. albicans to verify the presence of denture stomatitis will be studied by Drs. D.R. McArthur and M.T. Wood at UNC and Dr. D.R. Nelson at the Durham VA Medical Center.

Enclosed also find a preliminary draft of Table 1 showing the positivity of subgingival plaque sites of HIV-seropositive and HIV-seronegative periodontal patients observed with our silver stain. This positivity was confirmed by the North Carolina Memorial Hospital culture. We also gave a preliminary estimate of the scores of the positive slides. As far as we know there have not been any publications on the efficacy of staining procedures for demonstrating the presence of C. albicans in subgingival plaque. There has been a paper by P.A. Murray et al. (in Perspectives on Oral Manifestations of AIDS, P.B. Robertson and J.S. Greenspan, eds., PSG Publishing, Littleton, Mass., 1988) presenting a significant difference in numbers of C. albicans cultured from sites of HIV-seropositive and HIV-seronegative patients.

We have included light micrographs of our staining procedure for showing P. carinii in lung sections of AIDS patients as well as the light and electron micrographs showing the presence of candida in subgingival plaque specimens from AIDS patients. We have many other EM's of P. carinii in lung sections and candida in subgingival plaques that we are not sending at this time.

The subgingival plaques of some of the AIDS patients have also been stained for the demonstration of proteolytic enzymes and acid phosphatase. These enzymes are active in the hyphal forms of the candida and could be contributing to the breakdown of the periodontium and the adjacent bone. Much

larger numbers of macrophages were generally noted in the plaques from the AIDS patients with our acid phosphatase stain than in the corresponding samples from other perio patients.

Enclosed also find a copy of the October 23 issue of the University of Louisville paper which discussed Beverly Giammara's participation in this contract. Also find two abstracts we submitted about our work under this contract for presentation at the 1990 International Association for Dental Research Meeting and reprints of some recent brief papers.

Sincerely,

Jacob S. Hanker

Jacob S. Hanker, Ph.D.
Professor
Director, Biomaterials
Biomedical Engineering, UNC

Beverly L. Giammara/PEY

B.L. Giammara, B.S., M.S.

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JSH/rbw

Enclosures

cc: Captain James Curtis Cecil, III
Captain Robert G. Walter

Table 1. SCORES* FOR CANDIDA ALBICANS OBTAINED AFTER SIGMA DIAGNOSTICS HT-100 SILVER STAINING OF SUBGINGIVAL PLAQUE SMEARS FROM DISEASED SITES IN AIDS OR OTHER PERIODONTAL PATIENTS

*Score (0 TO 3)	8 HIV-seropositive patients (32 sites)	19 HIV-seronegative patients (24 sites)
0	4	15
1	7	3
2	11	6
3	10	0
	0 of the 8 HIV-seropositive patients had a score of 0 at all sites	13 of the 19 HIV-seronegative patients had a score of 0 at all sites

*Davenport Index:

0 - no candida seen on smear
1 - a few scattered organisms present
2 - large numbers present in a few fields
3 - large numbers present in most fields